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Biomolecular transport on silicified mammalian cells

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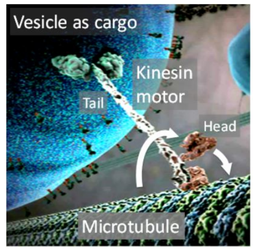
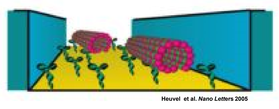
Introduction

- Microtubules (MTs) are cytoskeletal protein filaments that provide mechanical support for the cell, and serve as "tracks" for motor proteins to transport organelles
- Kinesin is a microtubule-based motor protein that "walks" along MTs by dissipating chemical energy, with a force of ~ 40pN nm and efficiency of ~ 50%[1]
- Kinesin-MT transport system has been used in many nanotechnological applications including biosensing[2], cargo transportation[3], and assembly of ring nanocomposites[4]




Heuvel et al. *Nano Letters* 2005

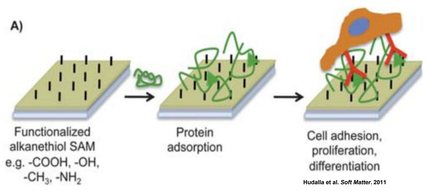
- MT guiding using lithographically nanostructured surfaces hinder MT motility and lead to MT loss[5]

Exploring alternative nanostructures is essential for reliable MT guiding

Cell patterning and preservation

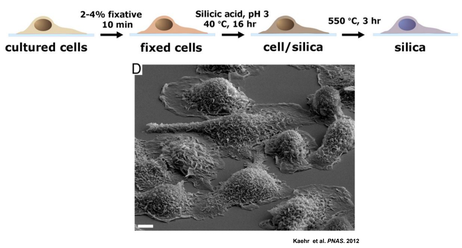
- Self-assembled monolayers (SAMs) previously used to modulate cell adhesion and spreading, allowing for size and shape control of cell patterns

  Limitation: environmental conditions render cells unstable for long-term applications



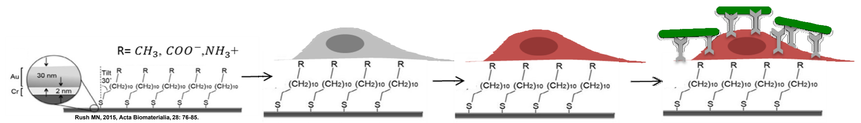
Hudalla et al. *Soft Matter*. 2011

- Preservation of cellular architecture through silicification process
  - Preserve user-defined 3D features
  - Provides simple alternative to specimen preparation and preservation (no expertise or specialized equipment needed)
  - Tolerate extreme environments



Kaehr et al. *PNAS*. 2012

Approach



SAMs with different functional groups are used to alter morphology of fibroblast cells

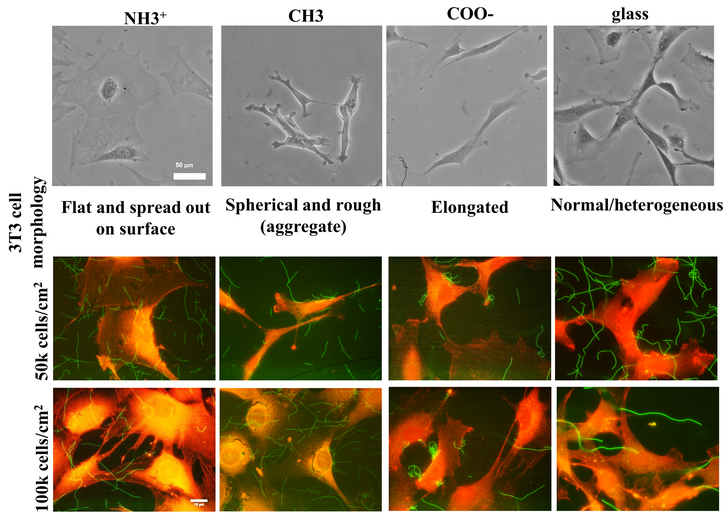
Cells cultured on substrates

Silicification of cells

Gliding motility assay- kinesin motors adhere to the silicified surface and translate MT filaments through ATP hydrolysis

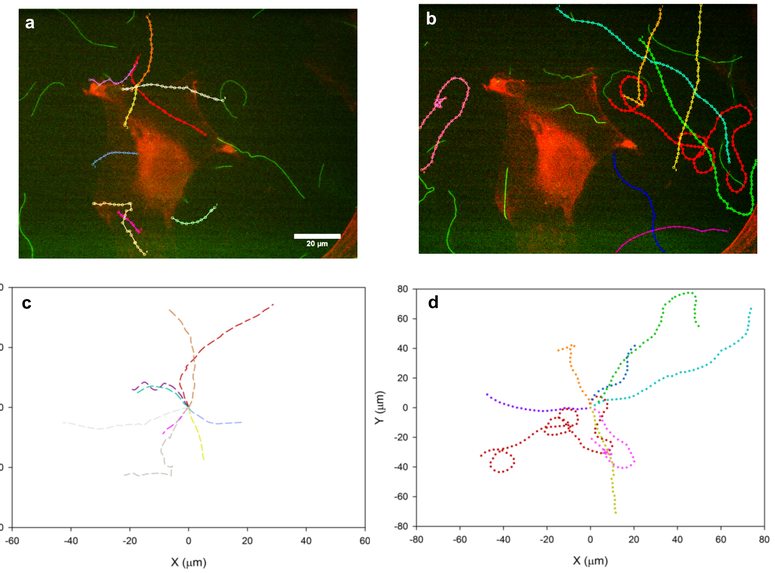
- Self-assembled monolayers (SAMs) on gold coated glass with chromium adhesion layer is used to alter mammalian cell morphology
- Cells are preserved through silicification process, and used as 3D nanostructures to explore the behavior of kinesin-MT system by implementing the gliding motility assay

Effect of SAMs and cell confluency on morphology and MTs



- NH_3^+ SAMs hindered MT motility at high and low cell density
- CH3 SAMs resulted in cells with poor adhesion to surface, where edge height prevented MTs from climbing (MTs got stuck on cell surface or changed direction)
- COO^- SAMs resulted in smooth gliding of MTs, however, MTs aggregated at edge of cells at high cell density

Microtubule trajectories and velocity



- MT gliding trajectories were evaluated using carboxyl (COOH) terminated SAMs and low cell count (50k cells/cm^2)
- MT trajectories were linear on silicified cells (a,c), while curved trajectories were observed on silicified SAMs surface (b,d)
- Gliding velocity remained constant with an average of 1 µm/s, independent of surface

CONCLUSIONS

- We established a unique "bottom-up approach" by combining well-established techniques to generate preserved, 3D biocompatible structures dictated by SAMs and cell confluency
- Preliminary experiments show promising results of surface topographies influencing MT translocation
- Future experiments will provide insight into the development of applications involving Kinesin-MT transport on complex nanostructures

References:

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